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## EUROPEAN PATENT APPLICATION

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(54) Disinfection process.

(57) Low molecular weight monoperoxyaliphatic acids such as peracetic acid are useful as broad spectrum biocides. An even broader spectrum of activity is achieved by selecting monoperoxyaliphatic acids having a carbon chain length of 6 to 9 atoms, which act as sporicides and in a wider pH range. A preferred biocide is peroxyheptanoic acid. The process can be carried out on solid surfaces such as in place equipment, or containers or in liquid media such as process or recirculating waters.

EP 0 233 731 A2

Disinfection Process

The present invention relates to disinfection and more particularly to the disinfection of surfaces or media employing a peroxygen compound as disinfectant.

There is a universal requirement for disinfectants that  
5 are effective against a broad spectrum of microorganisms.  
Thus, such disinfectants would be welcomed domestically for  
disinfecting toilets, work surfaces, sinks and drains in  
horticulture for disinfecting glass houses, pots and in  
farming for disinfecting animal or poultry houses or milking  
10 parlours in industry and especially the food and drinks  
industry for disinfecting of plant and apparatus in place,  
of cartons, canisters or containers, and process water or  
cooling fluid, especially if it recirculated, and in  
hospitals and other large-scale users of recirculating water  
15 for space heating. It has increasingly been recognised that  
low molecular weight monoperoxyaliphatic acids, and  
especially peroxyacetic acid, are broad spectrum biocides  
that have a wide applicability against bacteria,  
particularly in vegetative form, and are usable against  
20 viruses, moulds and yeasts. Accordingly there has been a  
significant increase in the extent of usage of peroxyacetic  
acid for disinfection, both as regards areas of use and  
amount employed. However, there is a continuing value in  
seeking further broad spectrum disinfectants that can either  
25 extend the conditions under which the disinfectant is  
effective or the microorganisms against which the  
disinfectant can be employed. It has been found that  
peroxyacetic acid is comparatively ineffective against  
bacterial spores and in alkaline conditions.

In addition, in the course of their investigations, the inventors of the instant invention have found that there are disadvantages inherent in the use of aliphatic monoperoxyacids that have a relatively high molecular weight, typified by monoperoxydecanoic acid(perlauric acid) which means that such peracids are less effective biocides than peracetic acid in use.

Surprisingly, it has now been found that a broad spectrum biocide that preserves its activity over a wider range of conditions than does peroxyacetic acid and is more effective against spores can be obtained by selection of aliphatic monoperoxyaliphatic acids having linear carbon chain length of 6-9 atoms.

The use peracids of the fatty acid series to provide fungicidal, bactericidal, antibrowning or color restoration treatment to plant tissue has been suggested in US Patent 2 512 640, but the specification does not describe or exemplify anywhere the selected range of peroxyacids of the instant invention, nor does it suggest anywhere that such a range could show the advantageous biocidal properties found by the inventors.

Accordingly, there is provided by the present invention a process for disinfecting solid surfaces or liquid media by bringing such an infected surface or medium into contact with an effective amount of a peroxyacid of formula  $R-CO_3H$  in which R represents an aliphatic hydrocarbon group having a carbon chain length of 5 to 8 atoms.

Advantageously, such peroxyacids show enhanced activity against a broad spectrum of microorganisms including spores and under alkaline conditions, whilst retaining effective activity under acid or neutral conditions.

It is often convenient to employ the invention disinfectant in the form of an aqueous solution, normally at a concentration of at least  $4 \times 10^{-4} M$  of peroxyacid and particularly selected in the range of  $1 \times 10^{-3}$  to  $1 \times 10^{-2} M$ . A saturated solution can be used. Particularly effective results have been obtained in the range of  $2 \times 10^{-3} M$  to

$5 \times 10^{-3} M$ . Whilst the solution can be employed at its natural, mildly acidic pH, it can be adjusted to be more acidic down to pH 3, suitably by the addition of a mineral acid or more alkali suitably up to pH 11, for example, by addition of an alkali, and particularly an alkali metal-based alkali such as sodium hydroxide, or sodium carbonate. One method of obtaining such solutions is by the introduction of solid preformed peroxyacid into solution.

The disinfectant can suitably be any of the linear monoperoxy aliphatic acids containing 6 to 9 carbon atoms, including specifically peroxyhexanoic acid, peroxyheptanoic acid, peroxyoctanoic acid and peroxynonanoic acid, and preferably is peroxyheptanoic acid in view of its manifest activity profile. Alternatively, the aliphatic chain can be non-linear, the compound preferably having a molecular weight of not more than 174. A suitable example of a non-linear peroxyacid is 3,5,5-trimethylperoxyhexanoic acid.

The process of disinfection can be carried by simply bringing the disinfectant into contact with the surface or media to be disinfected and maintaining contact for a brief period. Very satisfactory results have been obtained using a contact period of 5 minutes. Some disinfection will occur with shorter contact periods, such as desirably from 30 seconds upwards, and often at least 1 minute. Longer contact periods can be tolerated and are at the discretion of the user. By way of example, the surface or media can be permitted to remain in contact with the peroxyacid disinfectant until it has lost all its peroxyacid active oxygen. Accordingly, the contact period may last several hours or even days, when conditions so permit. The contact may be by a single shot addition of the peroxyacid or by periodic or slow constant addition such as to a bath or recirculating liquor. It will be recognised that if the medium to be disinfected is itself an aqueous medium, it can be treated by direct introduction of the selected peroxyacids whilst maintaining the solution pH within the range given previously herein.

The invention process can be carried out over a wider range of temperatures, for example from 0° to 100°C. Accordingly, the process can in many instances be effected without any change to the temperature of the substrate or media before treatment. Likewise the process can be readily carried out under the usual range of ambient temperatures encompassing both winter and summer conditions and conditions both indoors and out of doors, such as often in the range 10 to 30°C.

10 The methods of applying the instant invention process to surfaces and media are in essence the same as have hitherto been suggested for the application of peroxyacetic acid for the same purpose. Thus, for example, for liquid media the disinfectant can be directly introduced therein, and permitted to dissolve, thereby controlling the growth of microorganisms in recirculating process or heating waters or in discharges to public water courses. For the treatment of fixed apparatus or plant or work surfaces, walls or the like, a solution of the disinfectant can be sprayed or poured onto the surface to be disinfected and small articles such as containers or canisters can be immersed or sprayed.

Advantageously, it has also been found that not only are the disinfectants very effect against a broad spectrum of mono-organisms but in addition they tend to smell less repulsive than peroxyacetic acid does. To that extent, therefore, they are more acceptable to users.

Having described the invention briefly, the following examples are given in greater detail to demonstrate the invention process.

30 The microorganisms used in the tests were as follows:-

- |       |   |
|-------|---|
| Ec    | <u>Escherichia coli</u> NCTC 8196 (faecal organism)               |
| Pa    | <u>Pseudomonas aeruginosa</u> ATCC 15442 (Urinary tract pathogen) |
| 35 Sa | <u>Staphylococcus aureus</u> ATCC 6538 (skin organism)            |
| Sf    | <u>Streptococcus faecalis</u> ATCC 10541 (faecal organism)        |

Ca Candida albicans ATCC 10231 (vaginal pathogen)  
Sc Saccharomyces cerevisiae ATCC 9763  
Bs Bacillus subtilis NCTC 10452 (spore former)

5 Each test was carried out by a standardised suspension method. Sub cultures of the stock cultures were cultured in an appropriate medium, separated therefrom by centrifugation washed with sodium sulphate solution and then resuspended in  
10 fresh sodium sulphate solution at a concentration of between  $10^8$  and  $3 \times 10^8$  cfu ml<sup>-1</sup> for asporogenous bacteria and between  $10^7$  and  $3 \times 10^7$  cfu ml<sup>-1</sup> for yeasts and B. subtilis determined photometrically by comparing the measurement with standard suspension concentrations. A solution of the biocide at  
15 twice its tested concentration was made in demineralised water within 60 minutes prior to the test and a 5.0ml portion transferred to a sterile test tube and diluted with 40.0ml demineralised water and 1.0ml of microbial suspension. Thus, in each test a solution of one of the microorganisms was dosed into the liquid biocidal solution to provide an  
20 initial concentration of about  $10^6$  to  $10^7$  cfu ml<sup>-1</sup>. The abbreviation "cfu" indicates "colony-forming units".

At the end of the test period of 5 minutes, the solutions containing biocide were neutralised as to biocidal activity by the addition of a CT neutraliser except for the  
25 combinations of peroxyheptanoic acid and Bs/Sc and peroxy-nonanoic acid and Pa/Sa/Sc/Bs when TTLC neutraliser was used. CT neutraliser consisted of a mixture by volume of 1 part of a catalase solution of Sigma C-10 catalase 2.5g in 1 litre demineralised water sterilised by membrane  
30 filtration, pore size  $4.5 \times 10^{-7}$  m and 9 parts of a solution of sodium thiosulphate pentahydrate (50g) in 1 litre of demineralised water sterilised at 121°C. The TTLC neutraliser was the same as the CT neutraliser except that  
35 the thiosulphate was dissolved in a mixture of a nonionic surfactant (TWEEN 80) 30ml and demineralised water 970ml, and additionally contained Sigma P-5638 (L-phosphatidylcholine) 20g.

The concentrations of viable cells in the inocula are obtained by the method of serial dilution in Ringer solution and followed by the conventional p. plate methods with incubation plates at 37°C (30°C for *S. cerevisiae* for 48 hours). The mean number of colonies is then counted. Identical incubation conditions are used for the neutralised solutions.

The results of the tests are presented in the Table below, in which PAA represents the comparison peroxyacetic acid, PLA the comparison peroxy lauric acid (peroxydodecanoic acid) PHA peroxyheptanoic acid and PNA peroxy nonanoic acid. The PLA comparison peroxyacid could be introduced at the concentration of only  $1.16 \times 10^{-3}$  moles per litre rather than the higher concentrations of the other peroxyacids because it was less soluble than them, and even then it was not completely dissolved. The extent to which the microorganism population was reduced is indicated by the figures given in the Table, which are expressed on a logarithmic scale (base 10) or by +++ showing that no microorganisms survived or --- showing that no significant reduction in population had occurred.



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The Table

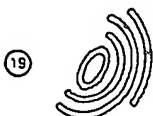
Micro-Organism	Biocide	Molar conc. x10 <sup>-</sup>	Population Reduction		
			pH5	pH7	pH9
5 Vegetative Bacteria					
Ec	PAA	2	+++	+++	+++
	PHA	2	+++	+++	+++
	PNA	2	+++	+++	+++
	PLA	1.16*	---	---	6
10 Pa	PAA	2	+++	+++	5
		4	+++	+++	5
	PHA	2	6	+++	+++
		4	+++	+++	+++
15	PNA	2	+++	+++	6
		2.5	+++	+++	+++
	PLA	1.16*	---	---	---
20 Sa	PAA	2	+++	+++	+++
	PHA	2	+++	+++	+++
	PNA	2	+++	6	+++
		2.5	+++	+++	+++
25 Sf	PLA	1.16*	4.3	5	5
	PAA	2	+++	+++	---
		4	+++	+++	+++
	PHA	2	+++	+++	+++
30	PNA	2	+++	+++	+++
	PLA	1.16*	6	4.6	4.3

	Micro-Organism	Biocide	Molar conc. $\times 10^{-3}$	Population Reduction		
				pH5	pH7	pH9
5	Yeasts					
	Ca	PAA	2	+++	---	---
			4	+++	+++	---
		PHA	2	+++	+++	+++
		PNA	2	+++	+++	+++
10		PLA	1.16*	4	---	---
	Sc	PAA	2	+++	+++	4
			4	+++	+++	4
		PHA	2	+++	+++	+++
15		PNA	2	+++	+++	+++
		PLA	1.16*	4	4.1	3.7
	Bacterial Spore					
	Bs	PAA	4	---	---	---
20		PHA	2	+++	+++	---
		PNA	2	5	---	---
			2.5	5	3.4	---
		PLA	1.16*	---	---	---

From the Table above, it can be seen that at the same molar concentration the peroxyacids PHA and PNA performed better than did the comparison biocides, PAA and PLA as regards to range of microorganisms and pH conditions under which they were able to be used. Not only can the invention biocides be used against gram positive and gram negative bacteria but also are more effective against yeasts and spores.

## CLAIMS

1. A process for disinfecting solid surfaces or liquid media by bringing such an infected surface or medium into contact with an effective amount of a peroxyacid characterised in that the peroxyacid has the formula  $R-CO_3H$  in which R represents an aliphatic hydrocarbon group having a carbon chain length of 5 to 8 atoms.
2. A process according to claim 1 characterised in that the peroxyacid is a linear  $C_6-C_{10}$  monoperoxyacid.
3. A process according to claim 2 characterised in that the peroxyacid is peroxyheptanoic acid.
4. A process according to any preceding claim characterised in that the peroxyacid is employed in an aqueous solution at a concentration of  $10^{-3}M$  to  $10^{-2}M$ .
5. A process according to any preceding claim characterised in that the peroxyacid is employed at a pH of from 3 to 11.
6. A process according to any preceding claim characterised in that the peroxyacid is brought into contact with spores.
7. A process for disinfection employing any novel feature or combination of features described herein.



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European Patent  
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# EUROPEAN SEARCH REPORT

0233731  
Application Number

EP 87 30 0980

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	FR-A-2 321 301 (HENKEL) * Claim 1 * -----		A 61 L 2/18 A 01 N 37/16
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			A 61 L A 01 N
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 01-06-1989	Examiner PELTRE CHR.
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

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Disinfection Process

The present invention relates to disinfection and more particularly to the disinfection of surfaces or media employing a peroxygen compound as disinfectant.

There is a universal requirement for disinfectants that  
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Thus, such disinfectants would be welcomed domestically for  
disinfecting toilets, work surfaces, sinks and drains in  
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farming for disinfecting animal or poultry houses or milking  
10 parlours in industry and especially the food and drinks  
industry for disinfecting of plant and apparatus in place,  
of cartons, canisters or containers, and process water or  
cooling fluid, especially if it recirculated, and in  
hospitals and other large-scale users of recirculating water  
15 for space heating. It has increasingly been recognised that  
low molecular weight monoperoxyaliphatic acids, and  
especially peroxyacetic acid, are broad spectrum biocides  
that have a wide applicability against bacteria,  
particularly in vegetative form, and are usable against  
20 viruses, moulds and yeasts. Accordingly there has been a  
significant increase in the extent of usage of peroxyacetic  
acid for disinfection, both as regards areas of use and  
amount employed. However, there is a continuing value in  
seeking further broad spectrum disinfectants that can either  
25 extend the conditions under which the disinfectant is  
effective or the microorganisms against which the  
disinfectant can be employed. It has been found that  
peroxyacetic acid is comparatively ineffective against  
bacterial spores and in alkaline conditions.

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In addition, in the course of their investigations, the inventors of the instant invention have found that there are disadvantages inherent in the use of aliphatic monoperoxyacids that have a relatively high molecular weight, typified by monoperoxydecanoic acid(perlauric acid) which means that such peracids are less effective biocides than peracetic acid in use.

Surprisingly, it has now been found that a broad spectrum biocide that preserves its activity over a wider range of conditions than does peroxyacetic acid and is more effective against spores can be obtained by selection of aliphatic monoperoxyaliphatic acids having linear carbon chain length of 6-9 atoms.

The use peracids of the fatty acid series to provide fungicidal, bactericidal, antibrowning or color restoration treatment to plant tissue has been suggested in US Patent 2 512 640, but the specification does not describe or exemplify anywhere the selected range of peroxyacids of the instant invention, nor does it suggest anywhere that such a range could show the advantageous biocidal properties found by the inventors.

Accordingly, there is provided by the present invention a process for disinfecting solid surfaces or liquid media by bringing such an infected surface or medium into contact with an effective amount of a peroxyacid of formula  $R-CO_3H$  in which R represents an aliphatic hydrocarbon group having a carbon chain length of 5 to 8 atoms.

Advantageously, such peroxyacids show enhanced activity against a broad spectrum of microorganisms including spores and under alkaline conditions, whilst retaining effective activity under acid or neutral conditions.

It is often convenient to employ the invention disinfectant in the form of an aqueous solution, normally at a concentration of at least  $4 \times 10^{-4}M$  of peroxyacid and particularly selected in the range of  $1 \times 10^{-3}$  to  $1 \times 10^{-2}M$ . A saturated solution can be used. Particularly effective results have been obtained in the range of  $2 \times 10^{-3}M$  to



5x10<sup>-3</sup>M. Whilst the solution can be employed at its natural, mildly acidic pH, it can be adjusted to be more acidic down to pH3, suitably by the addition of a mineral acid or more alkali suitably up to pH 11, for example, by addition of an alkali, and particularly an alkali metal-based alkali such as sodium hydroxide, or sodium carbonate. One method of obtaining such solutions is by the introduction of solid preformed peroxyacid into solution.

The disinfectant can suitably be any of the linear monoperoxy aliphatic acids containing 6 to 9 carbon atoms, including specifically peroxyhexanoic acid, peroxyheptanoic acid, peroxyoctanoic acid and peroxy-nonanoic acid, and preferably is peroxyheptanoic acid in view of its manifest activity profile. Alternatively, the aliphatic chain can be non-linear, the compound preferably having a molecular weight of not more than 174. A suitable example of a non-linear peroxyacid is 3,5,5-trimethylperoxyhexanoic acid.

The process of disinfection can be carried by simply bringing the disinfectant into contact with the surface or media to be disinfected and maintaining contact for a brief period. Very satisfactory results have been obtained using a contact period of 5 minutes. Some disinfection will occur with shorter contact periods, such as desirably from 30 seconds upwards, and often at least 1 minute. Longer contact periods can be tolerated and are at the discretion of the user. By way of example, the surface or media can be permitted to remain in contact with the peroxyacid disinfectant until it has lost all its peroxyacid active oxygen. Accordingly, the contact period may last several hours or even days, when conditions so permit. The contact may be by a single shot addition of the peroxyacid or by periodic or slow constant addition such as to a bath or recirculating liquor. It will be recognised that if the medium to be disinfected is itself an aqueous medium, it can be treated by direct introduction of the selected peroxyacids whilst maintaining the solution pH within the range given previously herein.

The invention process can be carried out over a wider range of temperatures, for example from 0° to 100°C. Accordingly, the process can in many instances be effected without any change to the temperature of the substrate or media before treatment. Likewise the process can be readily carried out under the usual range of ambient temperatures encompassing both winter and summer conditions and conditions both indoors and out of doors, such as often in the range 10 to 30°C.

The methods of applying the instant invention process to surfaces and media are in essence the same as have hitherto been suggested for the application of peroxyacetic acid for the same purpose. Thus, for example, for liquid media the disinfectant can be directly introduced therein, and permitted to dissolve, thereby controlling the growth of microorganisms in recirculating process or heating waters or in discharges to public water courses. For the treatment of fixed apparatus or plant or work surfaces, walls or the like, a solution of the disinfectant can be sprayed or poured onto the surface to be disinfected and small articles such as containers or canisters can be immersed or sprayed.

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Having described the invention briefly, the following examples are given in greater detail to demonstrate the invention process.

The microorganisms used in the tests were as follows:-

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5 Each test was carried out by a standardised suspension method. Sub cultures of the stock cultures were cultured in an appropriate medium, separated therefrom by centrifugation washed with sodium sulphate solution and then resuspended in fresh sodium sulphate solution at a concentration of between  
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35 surfactant (TWEEN 80) 30ml and demineralised water 970ml, and additionally contained Sigma P-5638 (L-phosphatidylcholine) 20g.

The concentrations of viable cells in the innocula are obtained by the method of serial dilution in Ringer solution and followed by the conventional p. plate methods with incubation plates at 37°C (30°C for *S. cerevisiae* for 48 hours). The mean number of colonies is then counted. Identical incubation conditions are used for the neutralised solutions.

The results of the tests are presented in the Table below, in which PAA represents the comparison peroxyacetic acid, PLA the comparison peroxy lauric acid (peroxydodecanoic acid) PHA peroxyheptanoic acid and PNA peroxy nonanoic acid. The PLA comparison peroxyacid could be introduced at the concentration of only  $1.16 \times 10^{-3}$  moles per litre rather than the higher concentrations of the other peroxyacids because it was less soluble than them, and even then it was not completely dissolved. The extent to which the microorganism population was reduced is indicated by the figures given in the Table, which are expressed on a logarithmic scale (base 10) or by +++ showing that no microorganisms survived or --- showing that no significant reduction in population had occurred.

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The Table

	Micro-Organism	Biocide	Molar conc. x10 <sup>-3</sup>	Population Reduction		
				pH5	pH7	pH9
5	Vegetative Bacteria					
	Ec	PAA	2	+++	+++	+++
		PHA	2	+++	+++	+++
		PNA	2	+++	+++	+++
		PLA	1.16*	---	---	6
10	Pa	PAA	2	+++	+++	5
			4	+++	+++	5
		PHA	2	6	+++	+++
15			4	+++	+++	+++
		PNA	2	+++	+++	6
			2.5	+++	+++	+++
		PLA	1.16*	---	---	---
20	Sa	PAA	2	+++	+++	+++
		PHA	2	+++	+++	+++
		PNA	2	+++	6	+++
			2.5	+++	+++	+++
		PLA	1.16*	4.3	5	5
25	Sf	PAA	2	+++	+++	---
			4	+++	+++	+++
		PHA	2	+++	+++	+++
		PNA	2	+++	+++	+++
		PLA	1.16*	6	4.6	4.3
30						

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	Micro-Organism	Biocide	Molar conc. x10 <sup>-3</sup>	Population Reduction		
				pH5	pH7	pH9
5	Yeasts					
	Ca	PAA	2	+++	---	---
			4	+++	+++	---
		PHA	2	+++	+++	+++
		PNA	2	+++	+++	+++
10		PLA	1.16*	4	---	---
	Sc	PAA	2	+++	+++	4
			4	+++	+++	4
		PHA	2	+++	+++	+++
15		PNA	2	+++	+++	+++
		PLA	1.16*	4	4.1	3.7
	Bacterial Spore					
	Bs	PAA	4	---	---	---
20		PHA	2	+++	+++	---
		PNA	2	5	---	---
			2.5	5	3.4	---
		PLA	1.16*	---	---	---

From the Table above, it can be seen that at the same  
 25 molar concentration the peroxyacids PHA and PNA performed  
 better than did the comparison biocides, PAA and PLA as regards the  
 range of microorganisms and pH conditions under which they  
 were able to be used. Not only can the invention biocides  
 be used against gram positive and gram negative bacteria but  
 30 also are more effective against yeasts and spores.

## CLAIMS

1. A process for disinfecting solid surfaces or liquid media by bringing such an infected surface or medium into contact with an effective amount of a peroxyacid characterised in that the peroxyacid has the formula  $R-CO_3H$  in which R represents an aliphatic hydrocarbon group having a carbon chain length of 5 to 8 atoms.
2. A process according to claim 1 characterised in that the peroxyacid is a linear  $C_6-C_{10}$  monoperoxyacid.
3. A process according to claim 2 characterised in that the peroxyacid is peroxyheptanoic acid.
4. A process according to any preceding claim characterised in that the peroxyacid is employed in an aqueous solution at a concentration of  $10^{-3}M$  to  $10^{-2}M$ .
5. A process according to any preceding claim characterised in that the peroxyacid is employed at a pH of from 3 to 11.
6. A process according to any preceding claim characterised in that the peroxyacid is brought into contact with spores.
7. A process for disinfection employing any novel feature or combination of features described herein.